

	P3			P2				P1			
Age (years)	7mo	2	3	2	3	4	5	5	6	7	8
NK Cells											
CD16/56 (%)	8 (3-17)	14 (4-23)	19 (4-23)	6 (4-23)	10 (4-23)	3 (4-23)	7 (4-26)	13 (4-23)	6 (4-26)	5 (4-26)	5 (4-26)
CD16/56 (#)	259 (200-1200)	963 (100-1000)	937 (100-1000)	244 (100-1000)	222 (100-1000)	128 (100-1000)	217 (90-900)	400 (100-1000)	116 (90-900)	113 (90-900)	107 (90-900)
T cell subsets (%)											
CD3+CD4+CD69+	--	--	--	--	1 (0-3)	--	--	--	0 (0-20)	--	--
CD3+CD4+CD25+	--	--	--	--	7 (7-25)	--	--	--	6 (7-27)	--	--
CD3+CD4+CD71+	--	--	--	--	8 (0-25)	--	--	--	8 (0-25)	--	--
CD3+CD4+CD40L+	--	--	--	--	1 (0-11)	--	--	--	2 (0-11)	--	--
CD3+CD4+CD134+	--	--	--	--	1 (1-5)	--	--	--	3 (1-5)	--	--
CD3+CD4+HLA ⁺	--	--	--	--	8 (0-15)	--	--	--	14 (0-15)	--	--
CD3+CD4+CD95+	--	--	--	--	41 (27-79)	--	--	--	54 (27-79)	--	--
CD4+TCRαβ	--	--	--	--	92 (88-100)	--	--	--	95 (88-100)	--	--
CD4+TCRγδ	--	--	--	--	8 (0-13)	--	--	--	5 (0-13)	--	--
CD8+TCRαβ	--	--	--	--	83 (79-100)	--	--	--	91 (75-100)	--	--
CD8+TCRγδ	--	--	--	--	17 (0-21)	--	--	--	9 (0-21)	--	--
T cell function											
CD40L unstim	--	--	--	--	--	--	--	--	0 (0-20)	--	--
CD40L stim	--	--	--	--	--	--	--	--	79 (79-96)	--	--
ICOS unstim	--	--	--	--	--	--	--	--	26 (0-29)	--	--
ICOS stim	--	--	--	--	--	--	--	--	90 (74-97)	--	--
IL2 soluble receptor (unit/mL)	--	999 (<2126)	--	--	1009 (<2126)	949 (<2126)	--	--	1007 (<2126)	1131 (<2126)	--

spontaneous proliferation (cpm)	--	--	135 (0-280)	--	72 (0-280)	--	130 (0-280)	--	250 (0-280)	--	308 (0-280)
PHA response (cpm)	--	--	226567 (\geq 135190)	--	332832 ($>$ 135190)	--	194114 (\geq 135190)	--	278167 ($>$ 135190)	--	290128 (\geq 135190)
CON A response (cpm)	--	--	307448 (\geq 75240)	--	228453 ($>$ 75240)	--	244807 (\geq 75240)	--	331152 ($>$ 75240)	--	309627 (\geq 75240)
PWM response (cpm)	--	--	72824 (\geq 26677)	--	60258 ($>$ 26677)	--	78628 (\geq 26677)	--	87798 ($>$ 26677)	--	97283 (\geq 26677)
Antibody response											
TTG IgA (CU)	--	--	--	--	2 (0-19)	--	--	--	<2 (0-19)	--	--
Diphtheria Ab (units/mL)	--	--	--	--	0.4	--	--	--	0.1	--	--
Tetanus titer (unit/mL)	--	--	--	--	1.1	--	--	--	0.6	--	--
Plasma cytokine levels											
IL-1B	--	<5 (\leq 25)	--	--	--	<5 (\leq 25)	--	--	--	<5 (\leq 25)	--
IL-2	--	2 (\leq 3)	--	--	--	2 (\leq 3)	--	--	--	3 (\leq 3)	--
IL-4	--	2 (\leq 8)	--	--	--	2 (\leq 8)	--	--	--	3 (\leq 8)	--
IL-5	--	1 (\leq 1)	--	--	--	1 (\leq 1)	--	--	--	1 (\leq 1)	--
IL-6	--	2 (\leq 6)	--	--	--	5 (\leq 6)	--	--	--	7 (\leq 6)	--
IL-8	--	11 (\leq 21)	--	--	--	4 (\leq 21)	--	--	--	4 (\leq 21)	--
IL-10	--	7 (\leq 6)	--	--	--	4 (\leq 6)	--	--	--	5 (\leq 6)	--
GM-CSF	--	1 (\leq 6)	--	--	--	1 (\leq 1)	--	--	--	1 (\leq 1)	--
IFN- γ	--	2 (\leq 5)	--	--	--	1 (\leq 5)	--	--	--	1 (\leq 5)	--
TNF- α	--	1 (\leq 7)	--	--	--	1 (\leq 7)	--	--	--	1 (\leq 7)	--

Table S1: Extended immune evaluations of STAT5B deficient siblings over time.

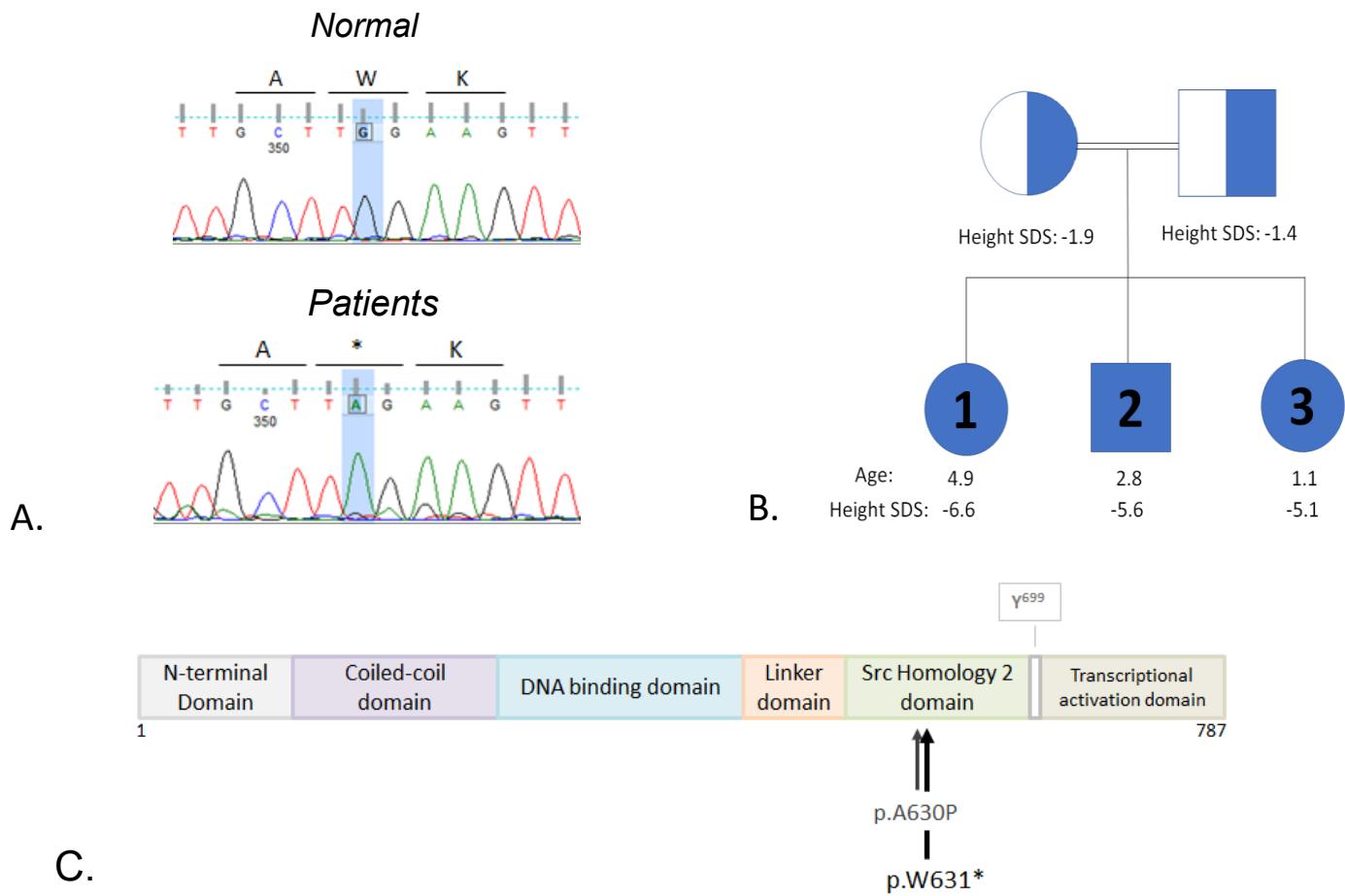


Figure S1: Identification of novel nonsense mutation in three siblings.

(A) Electropherogram of DNA sequences from a portion of exon 15 in a control sample compared to the siblings. The highlighted segment indicates the altered nucleotide. A, Ala; W, Trp; K, Lys; *, stop codon. (B) Pedigree of the identified family including the height SDS of each member. Half-filled shapes indicate heterozygous carriers. Completely-filled shapes indicate homozygous carriers. (C) Schematic representation of functional protein domains of STAT5B and approximate location of the identified patients' mutation alongside the first identified STAT5B mutation.

Gene	Zygosity	Variant	Variant Location	Significance	Phenotype
ARNT2	Homozygous	c.32-6678T>C	Intron	VUS	Webb-Dattani syndrome
THSR	Heterozygous	Rs6172289	Intron	Likely benign	AR hypothyroidism
TPO	Heterozygous	c.1352A>G	Exon	VUS	AR hypothyroidism

Table S2: Variants identified through WES analysis. These variants are filtered based on quality, frequency, genomic position, protein effect, pathogenicity and previous associations with the phenotype. In addition to STAT5B, 3 other variants were identified through filtering analysis. Data acquired through Sengenics analysis. VUS- variant of unknown significance. AR- autosomal recessive

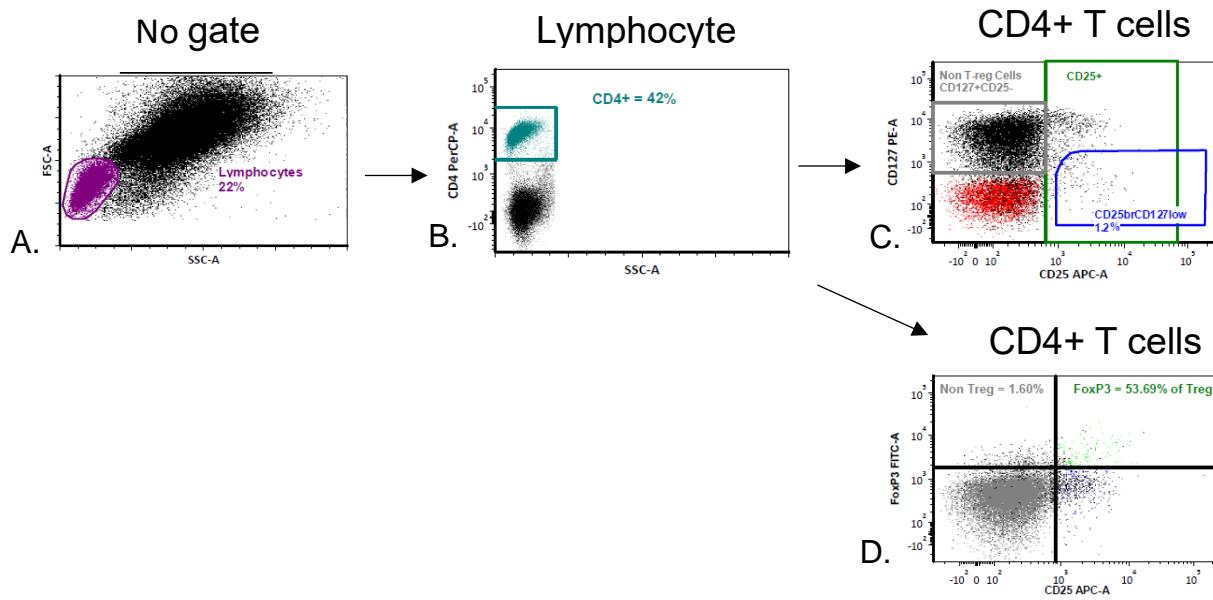


Figure S2: Flow schema for Treg evaluations in P1 at age 8. (A) Lymphocyte gating using scatter on PBMCs. (B) CD4 T cell specialization in lymphocyte subset. (C) CD25 and CD127 expression in CD4⁺ T cell subset. (D) CD25 and FOXP3 expression in CD4⁺ T cell subset.

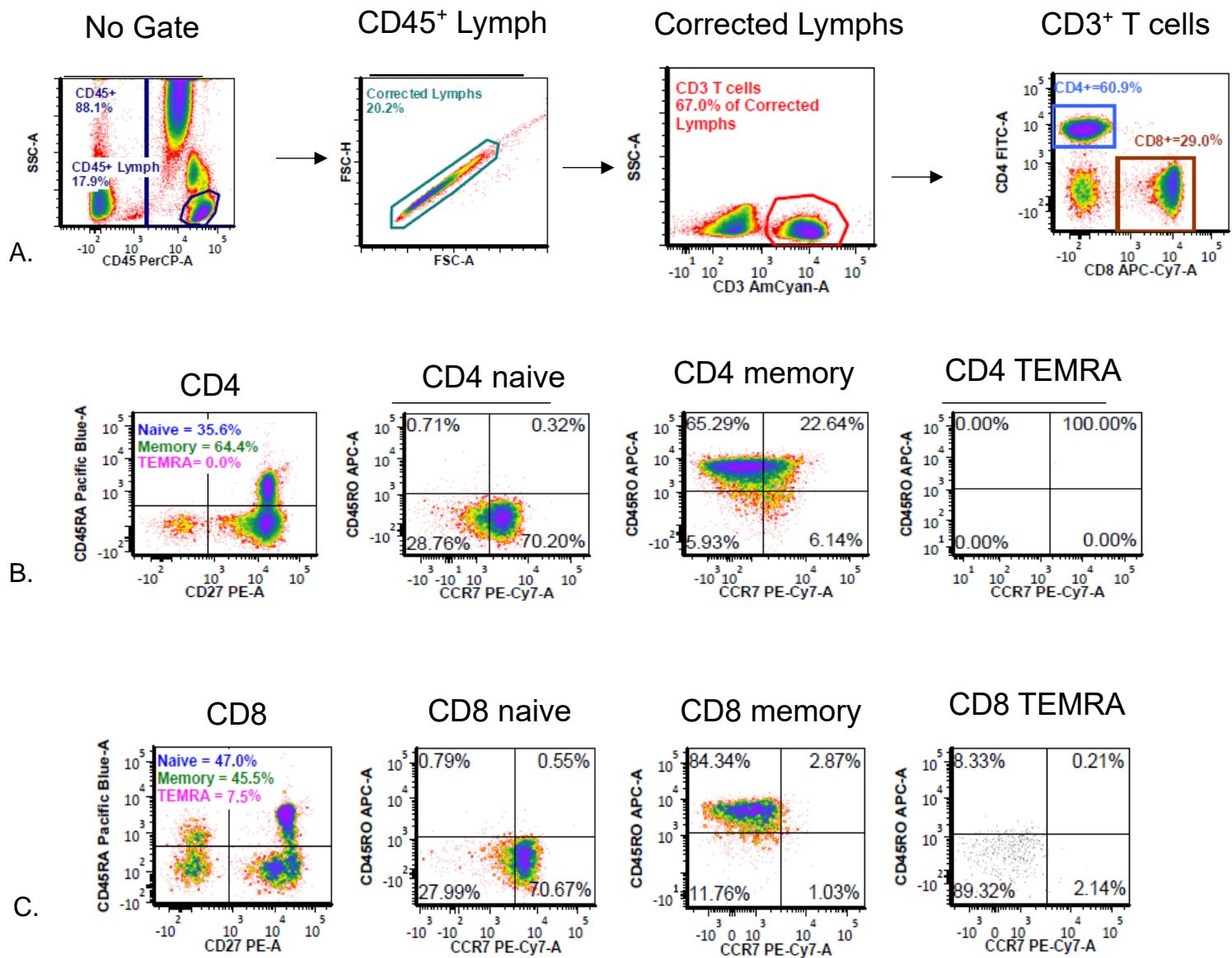


Figure S3: Flow schema of CD4⁺ and CD8⁺ naïve and memory T cell subsets in P1 at age 8. (A) Gating schema for lymphocytes and CD3⁺ T cells. (B) Flow sorting of CD4⁺ naïve and memory T cell subsets. (C) Flow sorting of CD8⁺ naïve and memory T cell subsets.

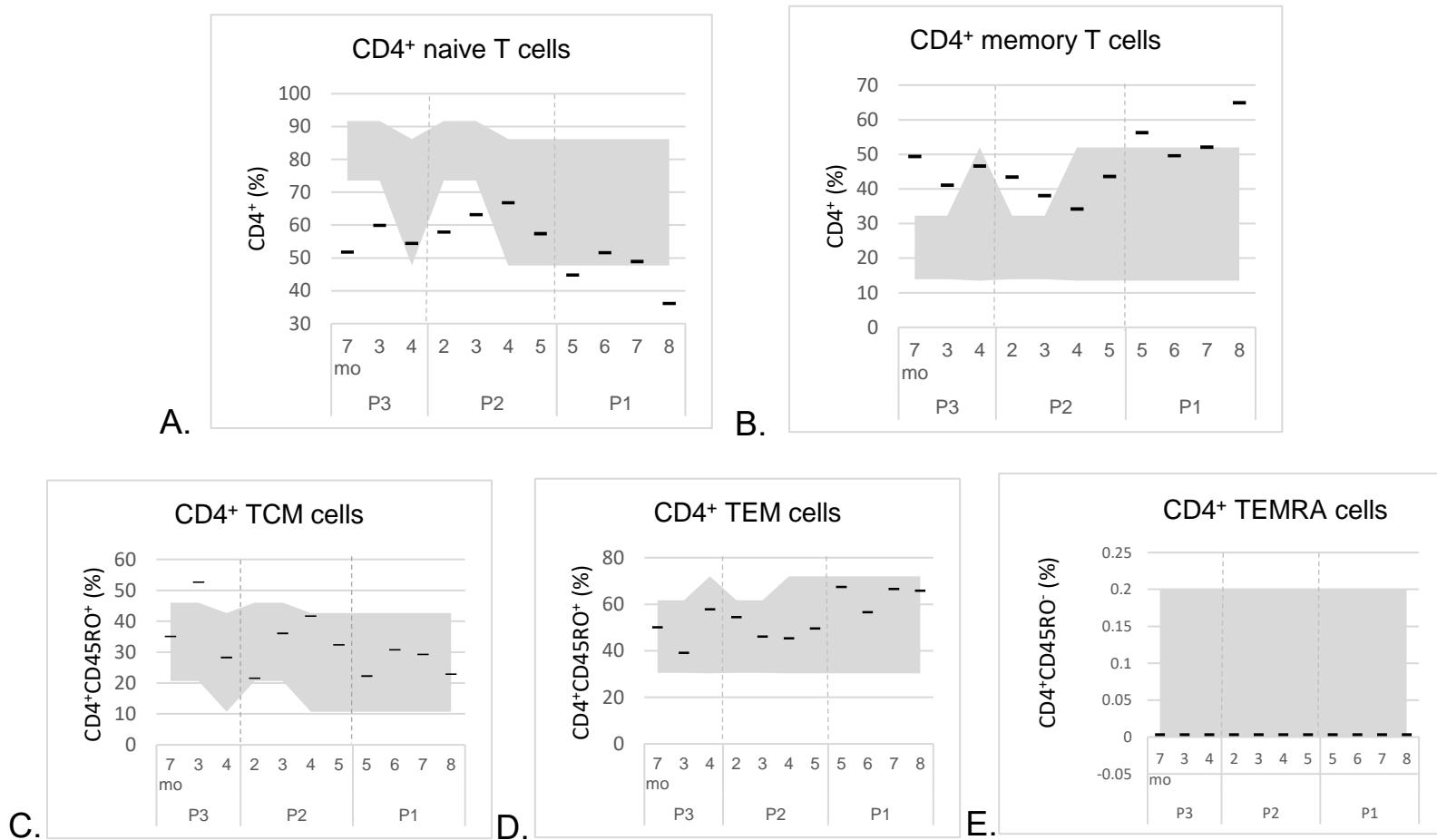


Figure S4: CD4⁺ naïve and memory T cell dysregulation in STAT5B deficient siblings over time.

(A) Percentage of CD4⁺CD45RA⁺ (naïve) T cells. (B) Percentage of CD4⁺CD45RO⁺ (memory) T cells. (C-E) Percentage of memory T cell subsets including (C) CD4⁺CD45RO⁺CCR7⁺ central memory T cells, (E) CD4⁺CD45RO⁺CCR7⁻ effector memory T cells, and (F) CD4⁺CD45RA⁺CCR7⁻ CD45RA re-expressing effector memory T cells. Grey area indicates normal range of T cell values.